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<b>(54) Title:</b> COMPOSITIONS CONTAINING POLY(HEXAMETHYLENE BIGUANIDE) SALTS AND USES THEREOF <b>(57) Abstract</b>  Uses of poly(hexamethylene biguanide) salts, especially the hydrochloride salt. Especially the use of the salts in the manufacture of medicaments for, and methods of topical treatment of microbial infections. Also pharmaceutical preparations, antiseptics, disinfectants and liquid formulations comprising a poly(hexamethylene biguanide) salt.		

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## COMPOSITIONS CONTAINING POLY(HEXAMETHYLENE BIGUANIDE) SALTS AND USES THEREOF

The present invention relates to poly(hexamethylene biguanide) salts, especially the hydrochloride salt (known as polyhexanide or PHMB), especially to the use of the salts in the manufacture of medicaments for, and methods  
5 of, topical treatment of microbial infections and to their use as antiseptics.

Microbial infections, such as bacterial, amoebal and fungál infections, are common and may be generalised, i.e. throughout the body, or may be localised, i.e. restricted  
10 to one area, for example, a wound site or an organ. (The term "microbes" is taken herein to include bacteria, amoebae, fungi and obligate intracellular organisms.)

Infections of the eye are relatively common and may be very serious, even sight threatening. At the present  
15 time there is a very limited number of agents that have been developed for use in the treatment of infections in the eye.

Currently the main antibiotics or antimicrobial agents used in the treatment of the eye are tetracycline,  
20 erythromycin, polymixin, trimethoprim, chloramphenicol, gentamicin, neosporin (a mixture of neomycin and bacitrasin), fusidic acid, quinolones and anti-fungal agents such as the azole group of agents. Propamidine, an antimicrobial, is often used as an over-the-counter (OTC)  
25 medication. Less commonly used are the sulphonamide group and beta-lactam antibiotics (e.g. penicillin). Although those agents are useful and safe, there is a continuing need for further antimicrobial agents which are safe for use in the eye.

30 Alternative agents for treatment are required which are less likely to produce microbial resistance and that are more effective, that is, have a broader spectrum and activity without problems of toxicity. These treatment

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compounds and compositions should be stable and easy to administer. Inexpensive treatments which fulfil these requirements are especially desired.

Polyhexanide is known to be a broad spectrum  
5 antibacterial agent. At relatively low concentrations the antibacterial action is biostatic and at higher levels, dependent on particular species of bacteria, it is bactericidal. It is a "membrane-active" antibacterial. Polyhexanide has been found to be active against both Gram-  
10 negative and Gram-positive bacteria.

Polyhexanide is currently sold as a swimming pool disinfectant, a water system disinfectant and at a concentration of approximately 0.00005%, as a preservative in contact lens solutions.

15 Polyhexanide has been used successfully in the treatment of Acanthamoeba keratitis, an uncommon but blinding amoebal infection of the cornea (the clear front surface of the eye), see, for example, Larkin et al., 1992, Hay et al., 1994, Seal 1994, Elder et al., 1994, and  
20 Bacon et al., 1993. The disease has a range of symptoms and resulting conditions including severe pain, ring abscesses, perforated corneas, corneal scarring and intumescent lens requiring keratoplasty, extracapsular lens extraction and posterior chamber lens implant. The  
25 infection can be difficult to treat and may be resistant to commonly used agents. Hence, polyhexanide was used in its treatment even though very little was known about the penetration of polyhexanide into the eye and what toxicity problems it might cause. In the treatment of Acanthamoeba  
30 keratitis polyhexanide has been recommended for use in very low concentrations: up to 0.02 % (w/v). In the treatment of an infection such as Acanthamoeba keratitis some relatively serious side effects of treatment may be acceptable when they would not usually be in the treatment  
35 of other, less serious, infections.

Various investigators have discussed and expressed

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concern about the toxicity of polyhexanide. For example, D. V. Seal in his paper in 1994 states that although polyhexanide has been applied topically in the treatment of Acanthamoeba keratitis it is not ideal for topical ocular treatment. Some toxic effects are discussed by Masaki Imayasu et al. 1992. Rabbit eyes were treated with sterilised phosphate buffered solutions of 0.01, 0.02 and 0.03 % polyhexanide and it was found, after fluorescein instillation, that there was superficial punctate staining on the cornea (showing lesions) and conjunctival chemosis.

It has, however, been found by the present inventors that polyhexanide can be used safely and without toxicity problems on the eye and in other locations.

The present invention is concerned with the use of any physiologically acceptable salt of poly(hexamethylene biguanide), for example, the hydrochloride, acetate or gluconate salt. Currently the only salt commercially available is the hydrochloride salt, which is known as "polyhexanide" or "PHMB". However, the invention is not limited to the use of the hydrochloride salt and, unless stated otherwise, references to polyhexanide or PHMB include all other physiologically acceptable salts.

The present invention provides the use of a poly(hexamethylene biguanide) salt, especially of poly(hexamethylene biguanide) hydrochloride, for the manufacture of a medicament for the topical treatment of microbial infection of the human or animal body, excluding amoebal infection of the eye.

The present invention especially provides the use of a poly(hexamethylene biguanide) salt, especially of poly(hexamethylene biguanide) hydrochloride, for the manufacture of a medicament for the topical treatment of microbial infection of the eye, excluding amoebal infection of the eye.

The present invention further provides use of a poly(hexamethylene biguanide) salt for the manufacture of a

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medicament for the topical treatment of microbial infection of the human or animal body, including infection of the eye, the salt being present in a concentration of at least 0.1 % w/v or w/w (w/v for solutions, w/w for other formulations).

It will be understood that the term "infection" does not imply the presence of a particular number of microbes; infection may be said to be present even if a relatively small number of microbes is present. The virulence of the organism, the host response and many intrinsic and environmental factors are all important factors.

The present invention also provides the use of a poly(hexamethylene biguanide) salt, especially poly(hexamethylene biguanide) hydrochloride, for the manufacture of an antiseptic for topical use on the human or animal body, including use on the eye.

The term "antiseptic", generally accepted, is used herein to have its generally accepted meaning of a preparation which is used for disinfecting, i.e. cleaning or sterilising, rather than for the treatment of infection. For example, an antiseptic may be used to clean the skin or the surface of the eye before surgery or may be used to clean a wound, made in surgery or in an accident, to prevent infection. Antiseptic preparations may be used in order to eradicate or prevent the spread of bacteria, amoeba or fungi or other microbes such as free obligate intracellular organisms. Polyhexanide when used as an antiseptic may also be able to kill certain free viruses (i.e. those coated in a cell membrane - enveloped viruses) even though it may not be a therapeutic antiviral agent.

Antiseptic preparations in accordance with the invention may also comprise further compounds having antimicrobial properties.

The present invention further provides a pharmaceutical preparation comprising a physiologically

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acceptable amount of a poly(hexamethylene biguanide) salt, especially poly(hexamethylene biguanide) hydrochloride, in a form suitable for topical administration in or on the human or animal body, excluding administration on the eye.

5 That is to say, the invention provides preparations of a type suitable for use in or on the body which are not suitable for application on the eye. Such preparation may, for example, comprise carriers or diluents which may not be used on the eye.

10 The present invention also provides a pharmaceutical preparation comprising a physiologically acceptable amount of a poly(hexamethylene biguanide) salt in a form suitable for topical administration in or on the human or animal body, including on the eye, the salt being present in a  
15 concentration of at least 0.1 % w/v or w/w (w/v for solutions, w/w for other formulations).

The present invention further provides an antiseptic preparation, comprising a physiologically acceptable amount of a poly(hexamethylene biguanide) salt, especially  
20 poly(hexamethylene biguanide) hydrochloride, in a form suitable for topical administration in or on the human or animal body, including use on the eye.

Preferably an antiseptic preparation comprises the poly(hexamethylene biguanide) salt in a concentration of  
25 at least 0.1 % w/v or w/w (w/v for solutions, w/w for other formulations).

The invention further provides a method of treating microbial infection, other than amoebal infection on the eye, comprising administering a therapeutically effective  
30 amount of a poly(hexamethylene biguanide) salt, especially poly(hexamethylene biguanide) hydrochloride, topically to the human or animal body.

The invention especially provides a method of treating microbial infection of the eye, other than an  
35 amoebal infection on the eye, comprising administering a therapeutically effective amount of a poly(hexamethylene

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biguanide) salt, especially poly(hexamethylene biguanide) hydrochloride, topically to the eye.

The invention also provides a method of treating a microbial infection comprising administering a  
5 therapeutically effective amount of a poly(hexamethylene biguanide) salt, especially poly(hexamethylene biguanide) hydrochloride, topically to the human or animal body, including to the eye, the salt being present in a concentration of at least 0.1 % w/v or w/w (w/v for  
10 solutions, w/w for other formulations).

The invention further provides a method of disinfecting (asepticizing) a part of the human or animal body comprising topical administration of an effective amount of a poly(hexamethylene biguanide) salt, especially  
15 poly(hexamethylene biguanide) hydrochloride, to the human or animal body, including to the eye. Preferably the salt is present in a concentration of at least 0.1 % w/v or w/w (w/v for solutions, w/w for other formulations)

The invention further provides for the use of a poly  
20 (hexamethylene biguanide) salt, especially poly (hexamethylene biguanide) hydrochloride, as a disinfectant for surfaces and for surface disinfectants which comprise a poly(hexamethylene biguanide) salt. Such a disinfectant may be used to disinfect medical equipment and medical and  
25 household surfaces. Disinfectants will usually comprise the salt in the form of an aqueous solution. The concentration of polyhexanide in a disinfectant will usually be relatively high and may be as high as 20 %. Preferably the concentration will be greater than 0.1 % w/v  
30 or w/w (w/v for solutions, w/w for other formulations).

Advantageously the antiseptic preparation, pharmaceutical preparation or method of treatment is in a form suitable for use on the skin; on or in a wound; on a mucosal surface, for example, in the nasal passages, the  
35 throat, in the bladder or in the vagina; as a colonic enema or lavage prior to or during surgery; in an ear cavity; in



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the mouth, for example, on the gums or on the teeth; on the scalp or on the hair.

Where the preparation is to be used to kill microbes on the skin surface or in a wound the preparation may take the form of an aqueous formulation, an oily formulation, an oil-in-water emulsion or a water-in-oil emulsion, a liposomal formulation or a gel formulation.

Where the preparation is to be used to kill microbes on mucosal surfaces the preparation may take the form of a mouth wash, a gargle, a slowly dissolving pastille or lozenge or a mouth, throat or nasal spray. A gel formulation may be, for example, suitable for application to the gums and both gel and paste formulations are suitable for use as toothpastes.

In a liquid formulation such a preparation may be used in bladder irrigation to eradicate or prevent the spread of infection.

For treatment of the hair, scalp or skin, shampoo or soap formulations of polyhexanide may be especially useful.

The treatment of microbial infections may be carried out in order to eradicate the bacteria, amoeba, fungi or other microbe completely or may be used to restrict or prevent the growth or spread of the bacteria, amoeba, fungi or other microbe.

Microbial infections which may be treated with polyhexanide include the common organisms which cause infection of the external eye and other parts of the body, such as, Gram positive cocci and rods, for example, Staphylococci species, Corynebacteria species and Streptococci species, Gram negative cocci and rods, for example, Haemophilus species, Pseudomonas species, Enterobacter species and Bacillus species, yeast forming fungi, for example, Candida, filamentary fungi, for example, Aspergillus and Fusarium, amoebae, for example, Acanthamoeba species, and obligate intracellular

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organisms, for example, Chlamydia species.

Common external conditions of the eye which may be treated are blepharitis, which is an infection of the eyelids and eyelid margins, conjunctivitis, which is an infection of the conjunctiva and the conjunctival sac, and keratitis, which is an infection of the clear cornea. In some cases a patient may suffer from all three infections at the same time.

The term "eye" is used herein to include not only the eyeball itself but also associated structures such as the eyelids, conjunctiva and conjunctival sac.

Administration of polyhexanide to the eye is to be topical administration. Use of the terms administration "to the eye" and "on the eye" are used to mean administration to the external surfaces of the eye. It is not intended that the polyhexanide be administered to internal tissues of the eye by means such as injection into the eye.

The invention also provides the use of a poly (hexamethylene biguanide) salt, especially poly(hexamethylene biguanide) hydrochloride, as a preservative in liquid formulations, for example, eye drops and contact lens solutions, for use on the eye, the polyhexanide being present in a concentration of greater than 0.01 %, preferably in the range of greater than 0.01 % to 2.0 % and more preferably in the range of greater than 0.01 % to 0.02 % w/v or w/w (w/v for solutions, w/w for other formulations; 1g per 100 ml of solution is 1% w/v).

When used therapeutically as an antimicrobial, polyhexanide will preferably be used in a concentration of at least 0.02 % and more preferably at least 0.1 %. Preferably the concentration will be in the range 0.02 % to 2.0 % and still more preferably in the range 0.1 % to 1.0 %. (Concentrations are calculated as w/v or w/w, w/v for solutions, w/w for other formulations.) When used as an

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antiseptic polyhexanide may be used in similar concentrations but will generally be used in higher concentration formulations.

Medicaments according to the present invention are provided in a pharmaceutical preparation form suitable for topical administration, for example, an emulsion, suspension, solution, cream, lotion, ointment, drops, foam, gel, a liposomal preparation, an aerosol or spray. Such preparations are generally conventional formulations, for example, as described in standard text books of pharmaceutics such as the British Pharmacopoeia or Martindale The Extra Pharmacopoeia.

Preparations for the treatment of eye infections may be in the form of aqueous formulations, oily formulations, liposomal formulations, gels or ointments. The polyhexanide may be mixed with other polymeric compounds such as polyacrylic acid. The pH of liposomal, aqueous and gel formulations may be adjusted to a pH in the range 6 to 8.5. Acid or alkali compounds may be used to adjust the pH, preferably with a buffer, especially a boric acid/borate buffer, a phosphate buffer or an acetic acid/acetate buffer.

Preparations for treatment of the eye may also comprise sodium chloride or another excipient to adjust the tonicity of the medicament.

Preparations, especially for treatment of the eye, may also comprise polyvinyl alcohol or another excipient, for example, hypromellose, to adjust the viscosity of the medicament.

Ointments, especially for treatment of the eye, may also comprise polyethylglycol, paraffin oils or other excipients to adjust the volume and viscosity of the medicament.

Liposomal preparations, especially for treatment of the eye, may comprise phosphatidylglycerol, phosphatidylcholine and/or chloresteryl dissolved in

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chloroform.

Gel formulations may comprise carbomer (polyacrylic acid) 940.

Antiseptic preparations according to the invention may take any of the forms discussed above. In addition antiseptic preparations may take the form of wipes, medicated plasters and other such forms which are commonly used for antiseptics.

Polyhexanide may be administered, for example, as a solution in physiological saline or in an artificial tear complex. An example of a solution which is suitable for use in the eye is one comprising polyhexanide and 0.3% hypromellose, 0.45% NaCl, 0.37% KCl, 0.19% borax (sodium tetraborate decahydrate) and 0.19% boric acid (% are w/v).

Further examples of formulations which may be used in accordance with the present invention are given in Examples 7 to 14.

The medicaments, and methods described herein may also comprise further pharmaceutically active substances, for example, steroids, such as Dexamethasone, antivirals, such as aciclovir and other compounds having antimicrobial activity, such as bacitracin and trimethoprim (currently used in Polytrim<sup>TM</sup> drops). The polyhexanide may be used in combination with other pharmaceutically active agents either in the same formulation or they may be applied in separate formulations.

The appropriate dose and frequency of treatment will be dependent on the symptoms and physical condition of the individual patient. In order to assist in determining the suitability of treatment with polyhexanide and also the optimal concentration and frequency of treatment samples of the infecting agent may be taken and grown in culture and those cultures subjected to sensitivity testing. Examples of such tests are described in Elder et al. 1994, Larkin et al. 1992 and Hay et al. 1994.

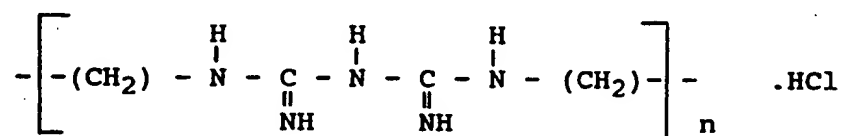
Medicaments administered to the eye are usually

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diluted by tears. It may therefore be advantageous to use relatively high concentrations of polyhexanide when treating the eye. In that way a therapeutically effective concentration of polyhexanide may remain in the eye for 5 longer.

Polyhexanide, especially in the form of drops, is suitable for general use as a first line therapy for external ocular infections such as blepharitis, conjunctivitis and keratitis. It may be used to replace 10 chloramphenicol, for which there are few alternatives, and may be especially useful in the U.S.A. where chloramphenicol is not used because of the rare, but fatal, complication of aplastic anaemia with which its use has been linked. It is cheap to manufacture and may therefore 15 be extremely valuable in treatment of patients in the Third World where corneal blindness from infection is prevalent. Apart from 5% Betadine<sup>TM</sup> (povidone iodine) there are no ocular antiseptics currently available commercially.

Polyhexanide is composed of a mixture of polymeric 20 compounds with the structure:



where n is generally in the range 2 to 30 inclusive with a 25 mean value of 5.5. If desired fractionation may be used to produce polyhexanide with a specific composition or mean value of n. The biguanide groups exist in the form of their salts so polyhexanide is essentially a polyelectrolyte. The hydrochloride salt is highly water 30 soluble.

The antibacterial activity of polyhexanide has been found to increase with chain length. This has been rationalised through the mechanism of action, see below;

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the longer chain being more effective in combining with acidic phospholipids such as phosphatidyl glycerol in the cell membrane. Advantageously, polyhexanide comprising a mixture of short and long polymer chains is used (see 5 Gilbert et al.).

Use of polyhexanide may overcome the drug resistance in bacterial infections since it seems unlikely that resistance to polyhexanide will develop, it will broaden the limited spectrum of currently used antibiotics and 10 cause fewer side effects. (Resistance is unlikely to develop to polyhexanide because of its mechanism of action: the polyhexanide attaches to the acidic phospholipids in the cell wall and disturbs the function of the membrane leading to leakage of intracellular contents.)

15 Although the primary use of the present invention is in the field of human medicine, it may also be desirable to treat other animals, especially mammals, for microbial infections, and the present invention therefore also includes uses of the described medicaments and preparations 20 for the treatment of animals other than humans and further includes preparations and medicaments described above that are suitable for the treatment of non-human animals.

The contents of the literature references mentioned herein are hereby incorporated by reference.

25 The following non-limiting Examples illustrate the invention. Examples 1 and 2 illustrate the efficacy of polyhexanide (PHMB) as an anti-microbial. Examples 3 to 6 illustrate the effect of polyhexanide (PHMB) on the ocular surface of the eye and confirm that it is safe for use at 30 the proposed concentrations. Examples 7 to 14 show a number of different formulations which are suitable forms in which to administer polyhexanide.

In Examples 1 to 6 the polyhexanide used was the hydrochloride salt. For Examples 7 to 14, the formulations 35 exemplified may comprise any physiologically acceptable salt of poly(hexamethylene biguanide) but preferably

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comprise the hydrochloride salt.

The Examples refer to the following Figures:

Figure 1 is a diagrammatic representation of the results of experiments described in Example 2. It is a graph showing the colony forming units/mL of a Pseudomonas aeruginosa clinical isolate in a test sample over time for a number of different treatment regimes with polyhexanide.

Figure 2 is a diagrammatic representation of the results of experiments described in Example 2. It is a graph showing the colony forming units/mL of NCTC 10662, a laboratory strain of Pseudomonas aeruginosa, over time for a number of different treatment regimes with polyhexanide.

Figure 3 is a diagrammatic representation of the results of experiments described in Example 4. It is a graph showing corneal thickness over time for a number of different experimental regimes.

#### Example 1 - Efficacy

The efficacy of polyhexanide as a bactericide has been tested and the results are set out below. The European Pharmacopoeia criteria for evaluation of antimicrobial activity for Ophthalmic Preparations are given in terms of the log reduction in the number of viable micro-organisms against the value obtained for the inoculum. The "A criteria" that are usually required to be passed are:

Organism	Criteria	Log Reduction				
		6h	24h	7d	14d	28d
<u>C.albicans</u> and <u>A.niger</u>	A			2		NI
	B				1	NI
<u>P.aeruginosa</u> and <u>S.aureus</u>	A	2	3			NR
	B		1	3		NI

35

NI:No Increase

NR:No Recovery

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The "A criteria" express the recommended efficacy to be achieved. In justified cases where the "A criteria" cannot be attained, for example, for reasons of an increased risk of adverse reactions, the "B criteria" (also set out above) must be satisfied. Polyhexanide eye drops of 0.01 and 0.02% concentrations, without preservatives, having the following compositions:

	polyhexanide	0.01 % or 0.02%
	hypromellose	0.3 %
10	sodium chloride	0.45 %
	potassium chloride	0.37 %
	borax	0.19 %
	boric acid	0.19 %
	purified water	to 100 %

15 were tested and the results were as follows.

Product	Storage	Organism	Log Reduction				
			6h	24h	7d	14d	28d
20 PHMB 0.01%	<25°C	<u>C.albicans</u>	NR	NR	NR	NR	NR
		<u>A.niger</u>	0.60	1.30	2.22	2.85	3.02
		<u>P.aeruginosa</u>	NR	NR	NR	NR	NR
		<u>S.aureus</u>	NR	NR	NR	NR	NR

Product	Storage	Organism	Log Reduction				
			6h	24h	7d	14d	28d
25 PHMB 0.02%	<25°C	<u>C.albicans</u>	NR	NR	NR	NR	NR
		<u>A.niger</u>	0.45	1.23	2.25	3.31	NR
		<u>P.aeruginosa</u>	NR	NR	NR	NR	NR
		<u>S.aureus</u>	NR	NR	NR	NR	NR



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As may be seen both of the solutions tested satisfied the A criteria.

Example 2 - Efficacy against a wide range of microorganisms

- 5       The minimum inhibitory concentration (MIC) of polyhexanide was found for a broad range of microbes. Those microbes, listed below with the MIC values, are all known ocular pathogens and may also be found on or in other parts of the body.
- 10       The experimental procedure used was the standard one used for calculating MIC values. The procedure is set out in the Clinical Microbiology Procedures Handbook (Isenberg 1992a).

      The microbes used in the tests were all stored  
15 clinical isolates. All MIC values are concentrations given in parts per million.

Gram positive organisms

- (a) Staphylococcus aureus  
14 isolates tested - mean MIC 3.5 ppm range 1 ppm to 20  
20 ppm
- (b) Coagulase negative Staphylococci  
8 isolates tested - mean MIC 2.5 ppm range 1 ppm to 5 ppm
- (c) Streptococci species  
14 isolates tested - mean MIC 2 ppm range 1 ppm to 5 ppm

25 Gram negative organisms

- (d) Pseudomonas aeruginosa  
17 isolates tested - mean MIC 15 ppm range 5 ppm to 35 ppm
- (e) Enterobacter species  
6 isolates tested - mean MIC 16.5 ppm range 5 ppm to 30  
30 ppm
- (f) Bacillus species  
2 isolates - MICs 10 ppm & 30 ppm

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Yeasts(g) Candida species

8 isolates tested - mean MIC 2 ppm range 1 ppm to 5 ppm

Fillamentous fungi5 (h) Aspergillus species

4 isolates tested - mean MIC 37.5 range 35 ppm to 40 ppm

(i) Fusarium

1 isolate tested - MIC 20 ppm

Figures 1 and 2 show the kill time for a range of 10 concentrations of polyhexanide against two isolates of Pseudomonas aeruginosa. One isolate is a clinical isolate taken from a patient (Figure 1) and the other, NCTC 10662, is a standard laboratory strain. The figures show plots of colony forming units/mL (cfu/mL) against time for microbe 15 populations treated with polyhexanide at the concentrations shown. The experimental procedure followed was the standard one used for kill time experiments, see the Clinical Microbiology Procedures Handbook (Isenberg, 1992b). (The controls used culture broth alone.)

20 MIC for the pseudomonas aeruginosa strain in Figure 1 is 30 ppm and for NCTC 10662 in Figure 2 is 7 ppm of PHMB.

Figures 1 and 2 show that PHMB has a very rapid antimicrobial action.

25 Example 3 - Toxicity

In these sets of experiments corneal hydration following direct application of a test solution to a whole eye was studied. The experiments were carried out in two sets:

- 30 (A) Whole eyes were used with the epithelium intact.  
(B) Whole eyes were used but with the epithelium completely removed prior to exposure to the test solutions.

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Fresh unscaled pig eyes were kept on ice until dissected, usually within 4 hours of slaughter. After measuring the corneal thickness using an ultrasonic pachymeter (Pachpen, Oculab, Mentor), the whole eye was placed face down in a deep dish and the corneal epithelial surface was covered with the test solution for one hour. After drug application (test solution) the corneal thickness was remeasured then the corneal scleral buttons were dissected from the eye. The buttons were then weighed (weight 1) and placed epithelial surface down, covered with medium 199 (Sigma) and incubated at 37°C for four to six hours. After incubation, the corneas were re-weighed (weight 2), air dried at room temperature for 24 to 48 hours and re-weighed dry (weight 3). That was Experiment Set A. The experiments were also performed using eyes that had the epithelium completely removed prior to exposure to the test solution, Experiment Set B.

Controls were exposed to balanced salt solution (BSS (Alcon Laboratories)) only (BSS control). Test solutions were made up from 20% stock solution (aqueous) of PHMB diluted with BSS. The percentage change in weight was calculated by subtracting the dry weight (weight 3) from each of the wet weights (weights 1 and 2) to give a pre-incubation net weight (weight 1 - weight 3) and a post-incubation net weight (weight 2 - weight 3) and dividing the post-incubation net weight by the pre-incubation net weight then multiplying by 100.

The percentage change represents the change in hydration of the eye tissues and gives an indication of changes in endothelial function, as does the change in corneal thickness. The greater the damage to endothelial function the greater the percentage change in weight.

Positive controls were performed in which the endothelial cells of the eye were destroyed after exposure to the test solutions thereby causing complete dysfunction of the endothelium. These are shown below as "+ control".

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For Experiment Set A only one positive control was carried out and that eye was treated with the BSS control solution. For Experiment Set B a positive control was performed with each of the four test solutions and with BSS control solution. The positive controls show the maximum change which would be measured if treatment with test solution had caused total dysfunction of the endothelium.

The results were as follows:

	Test solution	change in thickness mm ( $\pm$ SD)	% change in weight ( $\pm$ SD)
10	<u>Set A - epithelium intact</u>		
	BSS control	0.026 mm ( $\pm$ 0.028)	16.5% ( $\pm$ 7.8)
	BSS + control		31.3% ( $\pm$ 10.8)
	2% PHMB	-0.011 mm ( $\pm$ 0.01)	10.1% ( $\pm$ 4.3)
15	0.2% PHMB	-0.02 mm ( $\pm$ 0.016)	2.7% ( $\pm$ 5.6)
	0.1% PHMB	0.006 mm ( $\pm$ 0.025)	0.2% ( $\pm$ 5.0)
	0.02% PHMB	-0.039 mm ( $\pm$ 0.036)	11.8% (10.0)
	<u>Set B - epithelium removed</u>		
	BSS control	0.289 mm ( $\pm$ 0.033)	6.9% ( $\pm$ 5.0)
20	BSS + control		29.0% ( $\pm$ 1.3)
	2.0% PHMB	0.057 mm ( $\pm$ 0.058)	10.1% ( $\pm$ 4.3)
	2.0% PHMB + control		12.8% ( $\pm$ 9.3)
	0.2% PHMB	0.072 mm ( $\pm$ 0.024)	22.8% ( $\pm$ 4.3)
	0.2% PHMB + control		45.0% ( $\pm$ 13.8)
25	0.1% PHMB	0.033 mm ( $\pm$ 0.05)	13.5% ( $\pm$ 7.6)
	0.1% PHMB + control		31.9% ( $\pm$ 14.9)
	0.02% PHMB	0.099 mm ( $\pm$ 0.077)	21.6% ( $\pm$ 29.3)
	0.02% PHMB + control		43.1% ( $\pm$ 7.7)

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Summary -Set A - intact epithelium

The corneal thickness diminishes slightly after treatment with PHMB rather than thickening slightly as with the control. There was no evident endothelial damage, the percentage change in weight was not significantly different from that observed for the control test solution, and no significant dose effect was discernible.

Set B - epithelium removed

The corneal swelling observed with the control test solution was less than that observed with the polyhexanide (PHMB) test solution, but no significant dose effect was discernible. There was no evidence of endothelial damage.

The difference between the results of Set A and Set B show that there is little penetration of the PHMB through the epithelium.

Example 4 - Toxicity

In this example experiments were carried out to study the effect of polyhexanide on corneal thickness (a measure of endothelial cell function). These experiments were carried out on isolated mounted corneas.

Fresh unscalded pig eyes were dissected within 4 hours of slaughter. After removing the epithelium, the corneal scleral button was mounted on an artificial anterior chamber and the endothelial surface was perfused with BSS Plus (Alcon Laboratories) at 23 cm H<sub>2</sub>O, 37°C, with the anterior chamber volume replaced every 1/2 hour. Serial measurements of corneal thickness were made with an ultrasonic pachymeter. The epithelial surface was kept moistened with BSS and was not allowed to dehydrate. Where appropriate, the test solution was used to bathe the epithelial surface continuously for 1 hour. (Perfusion of the endothelium with BSS Plus was continued throughout). The test solution was made up from 20% stock solution (aqueous) of PHMB diluted

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with BSS.

Positive controls were produced by exposing the endothelium to 70% alcohol for 1 to 2 minutes and then continuing perfusion with BSS Plus. The positive controls  
5 show the results which would be obtained if complete dysfunction of the endothelium were to have been caused by the test solutions.

The results of these experiments are shown in Figure 3. Measurements of the corneal thickness were taken over a  
10 period of six hours. Figure 3 shows corneal thickness against time for that period.

Measurements for three control cornea are shown (control); these were cornea which were continuously perfused with BSS Plus for the full six hours and had their epithelial  
15 surfaces kept moistened with BSS. They received no other treatment. Measurements for a positive control are shown ("+" control). That cornea was treated as a normal control but the endothelial surface was exposed to 70% alcohol for 1 to 2 minutes after 120 minutes of the test period had passed.

20 Measurements for a cornea which had the epithelial surface bathed in 0.2% PHMB solution are shown (0.2% PHMB). Exposure to PHMB took place between minutes 120 and 180 of the experiment. The endothelium was perfused with BSS Plus for the entire six hour period. Measurements were also taken  
25 for a cornea receiving exposure of the epithelial surface to 0.2% PHMB solution for 1 hour followed by exposure of the endothelium to 70% alcohol for 1 to 2 minutes. This is shown as "0.2% PHMB "+" control" on Figure 3.

The results show that the 0.2% PHMB solution reduced  
30 corneal thickness, but there was no evidence of endothelial damage.

#### Example 5 - Toxicity

A series of experiments was carried out in which the effect of polyhexanide on cornea was studied by electron  
35 microscopy.

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Fresh unscaled pig eyes were placed in deep dishes and the epithelial surfaces were exposed to test solutions for 5 minutes. The test solutions were 2.0%, 0.2% and 0.02% PHMB solutions. Test solutions were made up from 20% PHMB stock solution (aqueous) diluted with BSS. BSS was used as the control solution. The corneal scleral buttons were then dissected and fixed in gluteraldehyde and processed for scanning electron microscopy.

Dose dependent changes in the morphology of the epithelium were seen but no discernible changes to the endothelium were observed (either when the epithelium was intact or removed). The 2.0% polyhexanide solution produced obvious dehydration of the epithelial cells with a large loss of cell volume and flattening of the cells, but no disruption of the cell membranes. The 0.2% polyhexanide solution produced an irregular but less complete loss of cell volume, producing an irregular appearance of the epithelial surface but not disruption of the cell membranes. The 0.02% polyhexanide solution produced changes that were similar but less pronounced than those produced by the 0.2% solution.

#### Conclusions from Examples 3, 4 and 5

Polyhexanide (PHMB) had an osmotic dehydrating effect on the cornea and limited penetration through the intact epithelium. There was some penetration of the PHMB into those cornea without epithelium but no endothelial damage was observed.

These results illustrate that polyhexanide may be safely used on the external eye and other epithelial surfaces of the body such as the skin and mucous membranes, at concentrations showing antimicrobial activity.

#### Example 6 - Toxicity

150 subjects with keratitis were treated with polyhexanide 0.02 % drops (composition as in Example 1) for an average of six months (range 1 to 18 months). Initial

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treatment of one drop per hour was given for 48 hours followed by 16 drops per day for one week and 4 to 6 drops a day thereafter. Although the subjects had large epithelial defects, no delay in corneal epithelial healing due to the drops was observed.

Two subjects developed punctate corneal epithelial erosions requiring therapy to be stopped after one month and another had punctate corneal epithelial erosions caused by the drops but was able to continue with the treatment. No other side effects of therapy were recognised and the drops were well tolerated by all other subjects. This rate of epithelial toxicity is mild compared with conventional treatment with propamidine and neomycin, which frequently cause punctate erosions of the cornea or inflammation of the conjunctiva (10 times more frequent than the PHMB treatment).

#### Examples 7, 8 and 9 - Aqueous Solutions

Compositions are given as % w/v.

##### (7) Phosphate buffered:

20	polyhexanide	0.1 to 2.0 %
	hypromellose	0.3 %
	sodium chloride	0.42 %
	sodium acid	0.075 %
	phosphate (2H <sub>2</sub> O)	
	magnesium chloride (6H <sub>2</sub> O)	0.03 %
25	sodium acetate (3H <sub>2</sub> O)	0.39 %
	acetic acid	0.001 %
	citric acid	0.00001465 %
	purified water	to 100 %

##### (8) Acetate/citrate buffered:

30	polyhexanide	0.1 to 2.0 %
	hypromellose	0.3 %
	sodium chloride	0.49 %
	potassium chloride	0.075 %
	calcium chloride (2H <sub>2</sub> O)	0.048 %



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	magnesium chloride (6H <sub>2</sub> O)	0.03 %
	sodium acetate (3H <sub>2</sub> O)	0.39 %
	acetic acid	0.001 %
	citric acid	0.00001465 %
5	purified water	to 100 %

## (9) Bicarbonate buffered:

	polyhexanide	0.1 to 2.0 %
	hypromellose	0.25 %
	sodium chloride	0.6 %
10	sodium bicarbonate	0.45 %
	purified water	to 100 %

Examples 10, 11 and 12 - Ointment formulations

Compositions are given as % w/w.

	(10) polyhexanide	0.1 to 2.0 %
15	yellow soft paraffin	to 100 %
	(11) polyhexanide	0.1 to 2.0 %
	liquid paraffin	30 %
	yellow soft paraffin	to 100 %
	(12) polyhexanide	0.1 to 2.0 %
20	polyethylene glycol 4000	to 100 %

Other ointments may be made by using different combinations and proportions of the substances listed in the three formulations given above.

Example 13 - Gel formulation

25 Compositions are given as % w/v.

	polyhexanide	0.1 to 2.0 %
	carbomer 940 polymer	0.2 %
	mannitol	5.0 %
	hydrochloric acid/	qs pH 7.4
30	sodium hydroxide	
	purified water	to 100 %

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Example 14 - Liposomal formulation

Compositions are given as % w/v

(phospholipid formulation)

	polyhexanide	0.1 to 2.0 %
5	phosphatidylglycerol	1.0 %
	phosphatidylcholine	4.0 %
	cholesterol	5.0 %
	chloroform	to 100 %

(See Surv. Ophthal. 1985;29;335-348.)

- 25 -

Bibliography

- Larkin, D.F.P., Kilvington, S. & Dart, J.K.G., Ophthalmology; Vol. 99; No. 2, 1992; 185-191, "Treatment of Acanthamoeba keratitis with polyhexanide biguanide".
- 5 Hay, John, Kirkness, Colin M. Seal, David V. & Wright, Peter Eye; Vol. 8; 1994; 555-563, "Drug resistance and Acanthamoeba keratitis; The quest for alternative antiprotozoal chemotherapy",
- Seal, D.V., BMJ, Vol. 308; 1994; 1116-1117, "Acanthamoeba  
10 keratitis"
- Elder, M.J., Kilvington, S. & Dart, J.K.G., Invest. Ophthalm. & Vis. Sci.; Vol 35; 1994; 1059-1064, "A Clinicopathologic Study of In Vitro Sensitivity Testing and Acanthamoeba keratitis"
- 15 Bacon, A.S., Frazer, D.G., Dart, J.K.G., Mattheson, M., Ficker, L.A. & Wright, P. Eye; Vol. 7; 1993; 719-725. "A Review of 72 consecutive cases of Acanthamoeba keratitis, 1984-1992"
- Masaki Imayasu, Takeshi Moriyama, Jun-ichi Ohashi, Hideji  
20 Ichijima, H. Dwight Cavanagh, Contact Lens Association of Ophthalmologists Journal; vol 18; October 1992; No. 4; 260-266
- Gilbert, Peter, et al., "Synergism within Polyhexamethylene Biguanide biocide formulations"; Journal of Applied  
25 Bacteriology; 69; 1990; 93-8.
- Isenberg, H.D. "Clinical Microbiology Procedures Handbook" American Society for Microbiology, Washington 1992,  
(a) Section 5, pp 5.2.1-5.2.29 (MICs) &  
(b) Section 5, pp 5.16.14-5.16.21 (Kill Times)

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Claims

1. Use of a poly(hexamethylene biguanide) salt for the manufacture of a medicament for the topical treatment of microbial infection of the human or animal body, excluding amoebal infection of the eye.
2. Use of a poly(hexamethylene biguanide) salt for the manufacture of a medicament for the topical treatment of microbial infection of the eye excluding amoebal infection of the eye.
3. Use of a poly(hexamethylene biguanide) salt for the manufacture of a medicament for the topical treatment of microbial infection of the human or animal body, including infection of the eye, the salt being present in a concentration of at least 0.1 % w/v or w/w (w/v for solutions, w/w for other formulations).
4. Use of a poly(hexamethylene biguanide) salt for the manufacture of an antiseptic for topical use in or on the human or animal body, including use on the eye.
5. A pharmaceutical preparation comprising a physiologically acceptable amount of a poly(hexamethylene biguanide) salt in a form suitable for topical administration in or on the human or animal body, excluding administration on the eye.
6. A pharmaceutical preparation comprising a physiologically acceptable amount of a poly(hexamethylene biguanide) salt in a form suitable for topical administration in or on the human or animal body, including on the eye, the salt being present in a concentration of at least 0.1 % w/v or w/w (w/v for solutions, w/w for other formulations).
7. An antiseptic preparation, comprising a physiologically acceptable amount of a poly(hexamethylene biguanide) salt in a form suitable for topical administration in or on the human or animal body, including on the eye.
8. An antiseptic preparation, comprising a

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physiologically acceptable amount of a poly(hexamethylene biguanide) salt in a form suitable for topical administration in or on the human or animal body, including on the eye, the salt being present in a concentration of at least 0.1 % w/v or w/w (w/v for solutions, w/w for other formulations).

9. A method of treating microbial infection, other than amoebal infection on the eye, comprising administering a therapeutically effective amount of a poly(hexamethylene biguanide) salt topically to the human or animal body.

10. A method of treating microbial infection of the eye, other than an amoebal infection on the eye, comprising administering a therapeutically effective amount of a poly(hexamethylene biguanide) topically to the eye.

11. A method of treating a microbial infection comprising administering a therapeutically effective amount of a poly(hexamethylene biguanide) salt topically to the human or animal body, including to the eye, the salt being present in a concentration of at least 0.1% w/v or w/w (w/v for solution, w/w for other formulations).

12. A method of disinfecting a part of the human or animal body comprising administering an effective amount of a poly(hexamethylene biguanide) salt topically to the human or animal body, including to the eye.

13. Use of a poly(hexamethylene biguanide) salt as a preservative in liquid formulations for use in the eye, the salt being present in a concentration of greater than 0.01% w/v or w/w (w/v for solutions, w/w for other formulations).

14. A use or a formulation as claimed in claim 13 wherein the salt is present in a concentration of up to 2.0 % w/v or w/w (w/v for solutions, w/w for other formulations).

15. A use or a formulation as claimed in claim 14 wherein the salt is present in a concentration of up to 0.02 % w/v or w/w (w/v for solutions, w/w for other

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formulations).

16. Use of a poly(hexamethylene biguanide) salt as a disinfectant for surfaces.

17. A disinfectant for surfaces which comprises a poly  
5 (hexamethylene biguanide) salt as an active agent.

18. A use, a composition or a method as claimed in any one of claims 1, 2, 5, 9 or 10 wherein the salt is present in a concentration of 0.02 % to 2.0 %, w/v or w/w (w/v for solutions, w/w for other formulations).

10 19. A use, a composition or a method as claimed in any one of claims 1, 2, 3, 5, 6, 9, 10 or 11 wherein the salt is present in a concentration of 0.1 % to 1.0 %, w/v or w/w (w/v for solutions, w/w for other formulations).

20. The invention as claimed in any one of claims 1 to  
15 19 wherein the poly(hexamethylene biguanide) salt is poly(hexamethylene biguanide) hydrochloride.

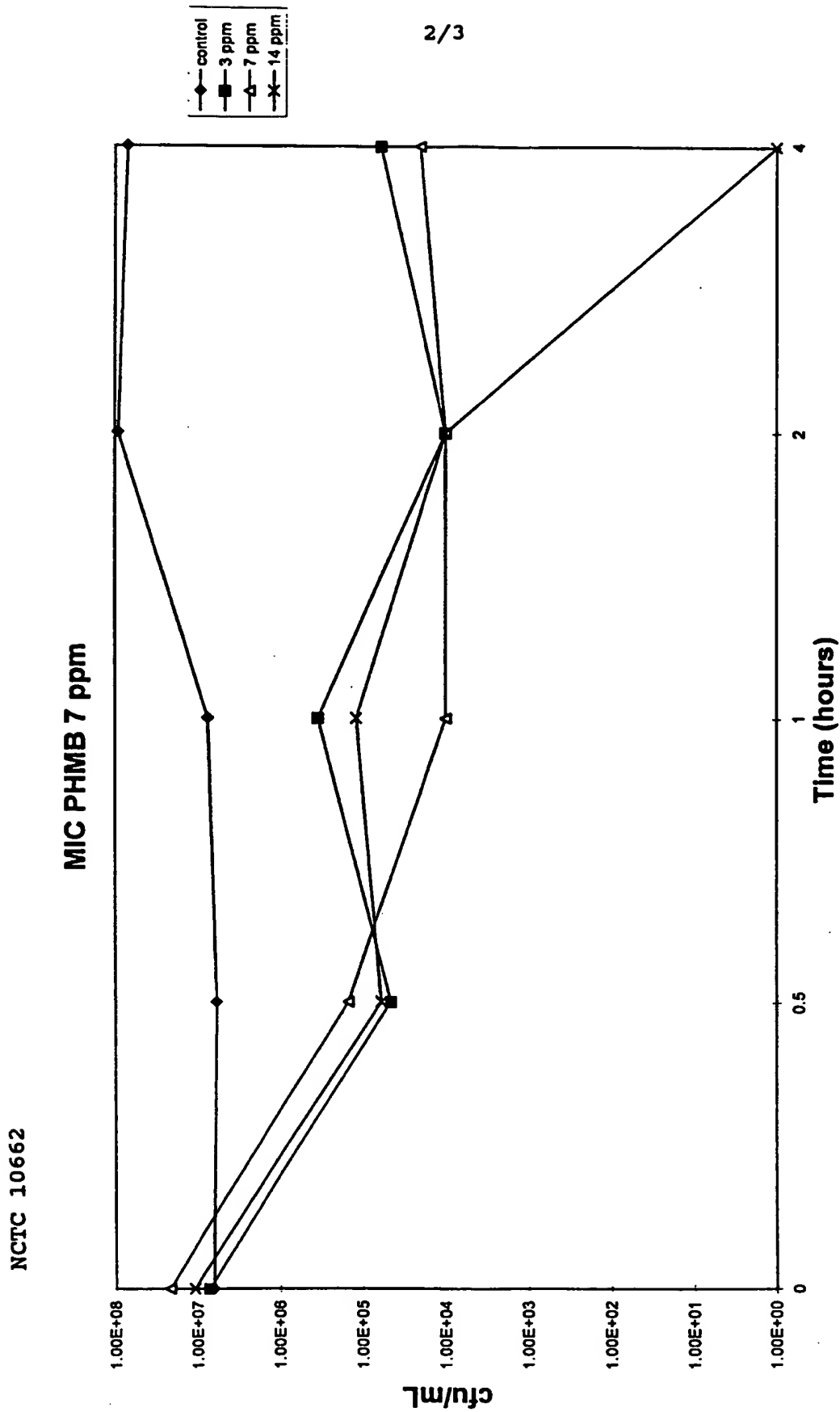


Figure 2

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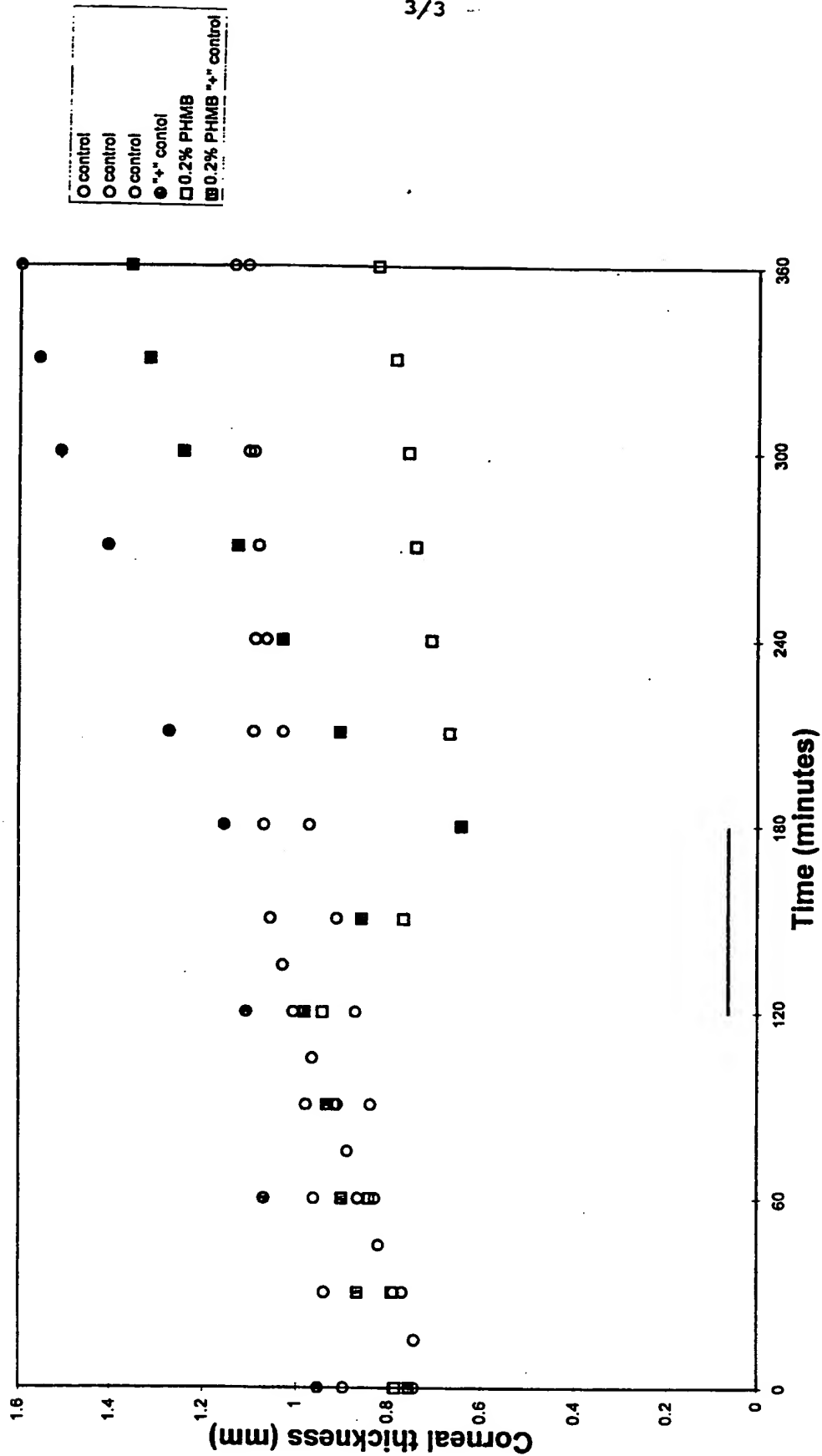


Figure 3



## INTERNATIONAL SEARCH REPORT

International Application No  
PCT/GB 96/01457

A. CLASSIFICATION OF SUBJECT MATTER  
IPC 6 A61K31/785 A61K31/155 A01N47/44

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 A61K A01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EP,A,0 468 122 (GUY ANDERMANN) 29 January 1992 * p.2, l.23-26; p.3, l.35-37; claims 1-9 *	1-20
X	HELV. ODONT. ACTA, vol. 17, no. 2, 1973, pages 105-8, XP000603388 H.W. TIKUS: "Topical gels containing chlorhexidine, vantocil, fluorophene and animal caries" * p.105, abstract; Table I *	1-9, 11, 12, 18-20
X	EP,A,0 450 117 (INFECTLESS SA ) 9 October 1991 see claims 1-9	1-9, 11, 12, 18-20

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

## \* Special categories of cited documents:

- "A" document defining the general state of the art which is not considered to be of particular relevance
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Date of the actual completion of the international search

14 October 1996

Date of mailing of the international search report

14. 11. 96

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## INTERNATIONAL SEARCH REPORT

International Application No

PCT/GB 96/01457

## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	OPHTALM. & VIS. SCI., vol. 35, 1994, pages 1059-64, XP000603790 ELDER ET AL: "A clinicopathologic study of in vitro sensitivity testing and Acanthamoeba keratitis" cited in the application * abstract; Tables 1-3; Discussion *	4,5,7, 13,14, 18-20
Y	see the whole document	1-3,6, 8-12,15
X	--- EP,A,0 293 761 (ALLPRO VENTURE OY ) 7 December 1988  see claims 1-4	1,2,4,5, 7,9,12, 16,17,20
X	--- WO,A,94 27440 (FRESENIUS AG ) 8 December 1994  * p.7, 1.29-30; p.8, 1.9-12; claims 1-12, 15-20 *	1-9, 11-15, 18-20
Y	--- DATABASE WPI Section Ch, Week 8836 Derwent Publications Ltd., London, GB; Class B04, AN 88-253361 XP002015831 & JP,A,63 183 502 (UENO SEIYAKU OYO KEN) , 22 June 1987 see abstract	1-20
Y	--- US,A,4 405 645 (RÖTHLISBERGER ET AL) 20 September 1983 see claims 1-5  -----	1-20

# INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/GB 96/01457

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP-A-468122	29-01-92	FR-A- 2644060	14-09-90
EP-A-450117	09-10-91	CA-A- 2039457	03-10-91
EP-A-293761	07-12-88	NONE	
WO-A-9427440	08-12-94	EP-A- 0700249	13-03-96
US-A-4405645	20-09-83	DE-A- 3012767	08-10-81
		GB-A,B 2074444	04-11-81
		JP-C- 1614935	15-08-91
		JP-B- 2038564	31-08-90
		JP-A- 56145213	11-11-81

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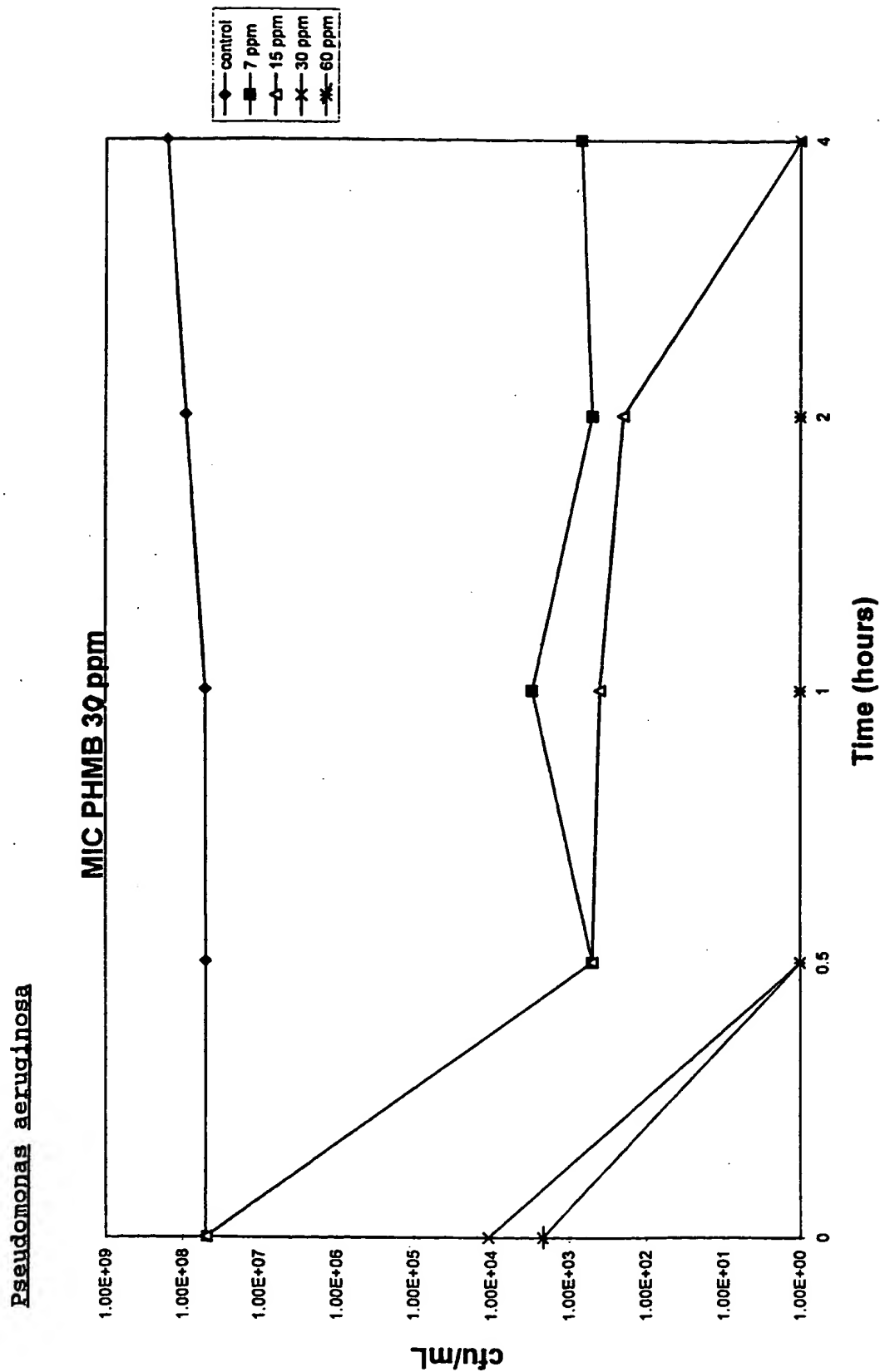


Figure 1